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SPECIES DIFFERENCE IN THE SPECIFIC RECEPTORS OF PLATELET ACTIVATING FACTOR

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Abstract—Relative potencies of platelet activating factor (PAF) and PAF analogs and several PAF receptor antagonists when inhibiting the [^{3}H]PAF specific binding to human and rabbit platelet membranes and membrane fragments of human lung tissues were compared. In rabbit platelets, L-652,731 was found to be most potent in the list of PAF receptor antagonists with an equilibrium inhibition constant (K_i) of 9.83 (±2.92) × 10^{-9} M followed by L-653,150 > kadsurenone ≈ Ono-6240 > ginkgolide B > CV-3988 > L-651,142, whereas in human platelets the relative potencies of these PAF receptor antagonists were as follows: Ono-6240 > L-653,150 ≈ L-652,731 ≈ kadsurenone > ginkgolide B > CV-3988 > L-651,142. Ono-6240 was the most potent one with a K_i of 4.86 (±1.44) × 10^{-8} M which was roughly two times more potent than that in rabbit platelets, whereas the affinity of L-652,731 was about ten times less in human platelets (K_i = 1.03 (±0.15) × 10^{-7} M) compared to that in rabbit platelets (K_i = 9.83 (±2.92) × 10^{-9} M). These variations between species among PAF antagonists strongly suggest that there exists a species difference at or near the binding site of the receptor of platelet activating factor. The relative potency of these PAF receptor antagonists in human lung membranes differed very little from that in human platelets and was found to be Ono-6240 > L-653,150 ≈ kadsurenone ≈ L-652,731 > ginkgolide B > CV-3988 > L-651,142. Even though C_{16}-PAF showed slightly higher potency in human lung, and CV-3988 and Ono-6240 showed slightly lower, the difference was too small to suggest that there is a difference in the PAF receptors between human platelets and human lung tissues.

Platelet activating factor (PAF†), derived from antigen-stimulated IgE sensitized rabbit basophils, is a potent lipid mediator with a myriad of biological activities [1–3]. Recently, its structure has been confirmed to be 1-*O*-alkyl-2-*O*-acetyl-sn-glycero-3-phosphocholine [4, 5]. Due to its high potency to activate platelets and its specific molecular structure requirements, a receptor-mediated process for the PAF function has been proposed. Indeed, specific receptor sites for PAF have been found in a variety of cell membranes including those from human [6, 7], dog [8] and rabbit platelets [9], human polymorphonuclear leukocytes [10] and plasma membranes of several types of smooth muscle cells [9, 11].

Several PAF antagonists have been reported recently [11–20, ‡]. With the availability of the published PAF antagonists and agonists and our own unpublished receptor antagonists, we were able to examine the relative potencies of these PAF receptor agonists and antagonists to inhibit the [^{3}H]PAF binding to human platelet membranes, rabbit platelet membranes, or membrane fragments of human lung tissues to determine the species difference and hopefully the tissue difference in the receptor of platelet activating factor.

MATERIALS AND METHODS

Materials. Tritium-labeled PAF (1-*O*-[1,2-$^3H_2$]alkyl-2-*O*-acetyl-sn-glycero-3-phosphocholine) was purchased from New England Nuclear with a specific activity of 45 Ci/mmole, in which 1-*O*-hexadecyl was >98% (NEN, Technical Service). Unlabeled C_{16}-PAF (1-*O*-hexadecyl-2-*O*-acetyl-sn-glycero-3-phosphocholine), C_{18}-PAF (1-*O*-octadecyl-2-*O*-acetyl-sn-glycero-3-phosphocholine) and enantio-C_{16}-PAF (3-*O*-hexadecyl-2-*O*-acetyl-sn-glycero-1-phosphocholine) were obtained from BACHEM (Torrance, CA). They were used in our assay without further purification.

Acetylamino-PAF (1-*O*-hexadecyl-2-deoxy-2-acetylamino-sn-glycero-3-phosphocholine) and azido-PAF (1-*O*-hexadecyl-2-deoxy-2-azido-sn-glycero-3-phosphocholine) were synthesized in Merck, and their chemical synthesis has been published [18].

Kadsurenone (Fig. 1) was isolated and purified from a Chinese herbal plant, heifenteng [19]. L-652,731 [*trans*-2,5-bis(3,4,5-trimethoxyphenyl)-tetrahydrofuran], L-653,150 [*trans*-2,5-bis(3,4,5-trimethoxyphenyl)tetrahydrothiophene] [21], Ono-

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† Abbreviations: PAF, platelet activating factor; BSA, bovine serum albumin; C_{16}-PAF, 1-*O*-hexadecyl-2-*O*-acetyl-sn-glycero-3-phosphocholine; C_{18}-PAF, 1-*O*-octadecyl-2-*O*-acetyl-sn-glycero-3-phosphocholine; acetylamino-PAF, 1-*O*-hexadecyl-2-deoxy-2-acetylamino-sn-glycero-3-phosphocholine; azido-PAF, 1-*O*-hexadecyl-2-deoxy-2-azido-sn-glycero-3-phosphocholine; and enantio-C_{16}-PAF, 3-*O*-hexadecyl-2-*O*-acetyl-sn-glycero-1-phosphocholine.

‡ T. Miyamato, H. Ohno, T. Yano, T. Okada, N. Hamanaka and A. Kawasaki, Kyoto Conference on Prostaglandins (Abstract), p. 99 (1984).

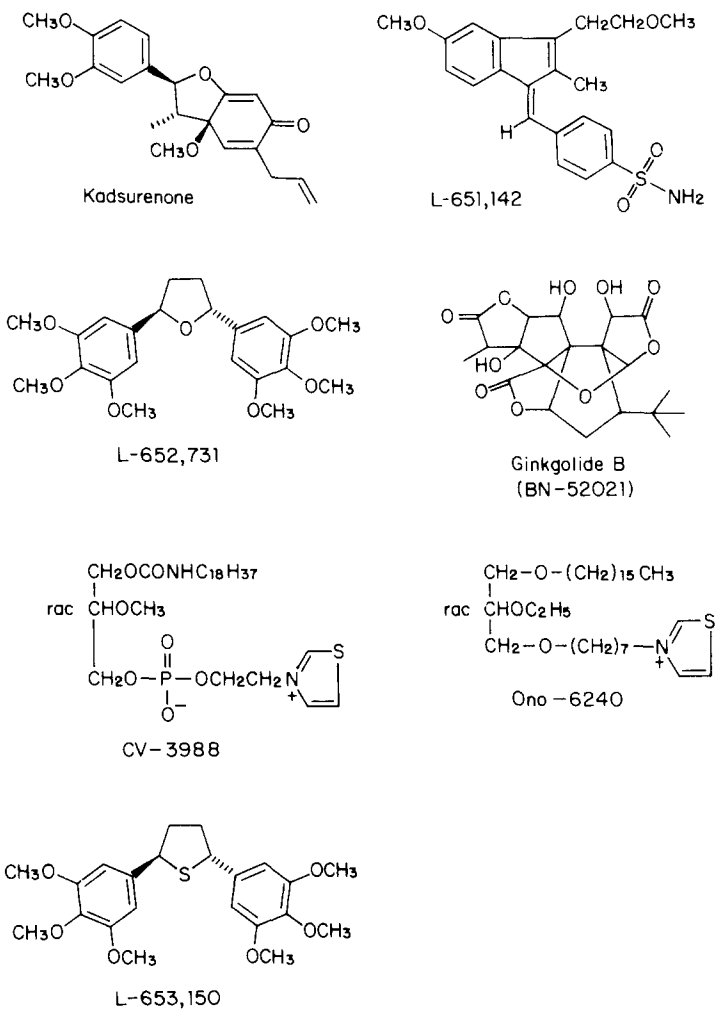

Fig. 1. Structural formulas of PAF-receptor antagonists.

6240 [1-*O*-hexadecyl-2*RS*-*O*-ethyl-3-*O*-(7-thiazolioheptyl)-glycerol chloride] CV-3988 [rac-3-(*N*-*n*-octadecyl-carbamoyloxy-w-methoxypropyl-2-thiazolioethyl phosphate] [12] and L-651,142 [E(*trans*) 1-[(4'-aminosulfonyl)-phenyl]-methylene-5-methoxy-2-methyl-1*H*-indene-3-(2'-methoxy) ethane] were synthesized in Merck for our on-going PAF project. Ginkgolide B (BN-52021), isolated from the Ginkgo biloba tree [16], was a gift from Dr. P. Braquet (Institute for Therapeutic Research, Le Plessis-Robinson, France).

Preparation of membrane fragments from rabbit platelets, human platelets, and human lung tissues. Human platelet membranes were prepared from platelet concentrates obtained from the New Jersey Blood Service (New Brunswick, NJ). After several washes with platelet wash solution (150 mM NaCl, 10 mM Tris, 2 mM EDTA, pH 7.5), the platelet pellets were resuspended in 5 mM $MgCl_2$, 10 mM Tris, and 2 mM EDTA at pH 7.0. The cells were then quickly frozen with liquid nitrogen and thawed slowly at room temperature. The freezing and thawing procedure was repeated at least three times. For further fractionation of membrane fragments, the lysed membrane suspension was layered over the top of a discontinuous sucrose density gradient of 0.25, 1.03 and 1.5 M sucrose prepared in 50 mM $MgCl_2$, 10 mM Tris and 2 mM EDTA, pH 7.0, and centrifuged at 63,500 *g* for 2 hr. The membrane fractions banding between 0.25 and 1.03 M (membrane A) and between 1.03 and 1.5 M (membrane B) were collected.

Rabbit platelets were prepared by the procedure already described in detail elsewhere [9]. Due to the optimal pH at 7.0 [15] and the inhibitory effect of Na^+ for the receptor binding assays [22], the rabbit platelets were finally suspended in Na^+-free medium containing 5 mM $MgCl_2$, 10 mM tris and 2 mM EDTA, pH 7.0, at a concentration of $2–5 \times 10^{-9}$ platelets/ml after being separated from plasma proteins and other blood cells through centrifugation at 4° as described before [9]. The platelets were then lysed by at least three cycles of freezing with liquid N_2 and thawing at room temperature. The membranes were further fractionated through the sucrose density gradient as described for human platelets.

Membranes of human lung tissues were prepared from the homogenates of normal human lung segments obtained from Memorial Sloan-Kettering Cancer Center (New York, NY) as previously described [11]. The thawed lung tissues from two or three individuals were cut into small pieces with scissors. The cut tissues were then homogenized with a polytron (Kinematica, GmbH, Switzerland) for 30 sec in 10 vol. (w/v) of 50 mM Tris, pH 7.6, at a setting of 5. The homogenate was filtered through two layers of gauge, and the filtrates were then centrifuged at 1,000 *g* for 10 min. The supernatant fraction was again centrifuged at 40,000 *g* for 15 min. The pellets were then resuspended in 5 mM $MgCl_2$, 10 mM Tris and 2 mM EDTA, pH 7.0, and further fractionated with a 10, 30, 35, 40 and 50% (w/w) discontinuous sucrose density gradient. The membrane fragments from each interface were collected and tested for [^{3}H]PAF specific binding as described below and for alkaline phosphatase activity as described [23]. The membrane fragments at the interface between 35 and 40% sucrose showed the highest [^{3}H]PAF binding and alkaline phosphatase activity.

The above prepared membranes were stored at −80° and thawed before use. No significant loss of [^{3}H]PAF specific binding was found during storage. The protein concentration of the membrane preparations was determined by the method of Lowry *et al.* [24] with bovine serum albumin (BSA) as the standard.

[^{3}H]*PAF receptor binding assay.* [^{3}H]PAF specific receptor binding to membranes of human platelets, human lung tissues, or rabbit platelets was performed as previously described [9, 25] with the following modification: the assays were done at optimal conditions at pH 7.0 [15] and in the presence of 10 mM $MgCl_2$ [22]. Unless otherwise specified, 100 μg membrane protein was added to a final 1-ml solution containing 1 pmole (1 nM concentration) of [^{3}H]PAF and a known amount of PAF or PAF receptor antagonists in 10 mM $MgCl_2$, 10 mM Tris and 0.25% BSA at pH 7.0. After a 2-hr incubation at 0°, the bound and unbound [^{3}H]PAF were then separated through a Whatman GF/C glass fiber filter under the house vacuum. No degradation of filter bound [^{3}H]PAF has been detected after 2 hr of incubation [17, 22]. The non-specific binding was defined as the total binding in the presence of excess unlabeled C_{16}-PAF (1 μM) where no further displacement was found with higher concentrations of C_{16}-PAF or PAF analogs or PAF receptor antagonists described here or other compounds already tested [25]. The specific binding was defined as the difference between total binding and non-specific binding. At a dose of 1 nM [^{3}H]PAF, the specific binding to human platelet and lung membranes varied between 35 and 50% of the total binding in the presence of 10 mM $MgCl_2$, whereas the specific binding in rabbit platelet membranes was 80–85% of the total binding.

To determine the relative potency among PAF, its analogs and its antagonists, the [^{3}H]PAF binding in the presence of inhibitors was normalized in terms of percent inhibition by assigning the total binding in the absence of inhibitors to be 0% inhibition and the total binding in the presence of 1 μM C_{16}-PAF to be 100%. Thus, the percent inhibition by the compound can be calculated by the formula expressed previously [14, 25]. The EC_{50} value was defined as the concentration of the inhibitor to obtain 50% inhibition of the specific [^{3}H]PAF binding. The IC_{50} values of receptor antagonists were determined more accurately from an indirect Hill plot [26], and the equilibrium inhibition constant (K_i) of the compound was calculated from the Cheng and Prusoff equation [27] as previously described [11, 17]. The equilibrium dissociation constants (K_D) used here to calculate K_i were 5.3×10^{-10}, 4.0×10^{-10}, and 4.9×10^{-10} M for rabbit platelets [22], human platelets (see Results), and human lung tissues [11] respectively.

RESULTS

Binding of [^{3}H]*PAF to human platelet membranes.* Figure 2A shows the binding of [^{3}H]PAF to one of the isolated human platelet membranes. Nonspecific binding was linear with the concentration of [^{3}H]PAF in the presence of excess (1000-fold) unlabeled C_{16}-PAF. The specific binding, defined as the total amount of [^{3}H]PAF bound minus the nonspecific binding, was saturable. It reached a maximum around 2–3 nM [^{3}H]PAF. Scatchard analysis, as shown in Fig. 2B, revealed the presence of a single class of receptor sites. As shown in Table 1, both membrane fractions A and B showed PAF specific binding, and the equilibrium dissociation constant from either membrane A or membrane B appeared

Table 1. Equilibrium dissociation constant (K_D) and the maximal number of PAF receptor sites (B_{max}) of several membrane preparations from human platelets

Membrane preparation	K_D (10^{-10} M)	B_{max} (moles/mg protein)
Memb #188A	4.83	2.864×10^{-13}
Memb #188B	4.66	5.123×10^{-13}
Memb #191A	3.22	8.187×10^{-14}
Memb #191B (Expt. 1)	2.50	3.172×10^{-13}
(Expt. 2)	5.44	3.370×10^{-13}
Memb #196A	4.59	1.834×10^{-13}
Memb #196B	4.34	1.01×10^{-13}
Memb #129B (Expt. 1)	4.25	2.96×10^{-13}
(Expt. 2)	4.57	2.50 ± 10^{-13}
(Expt. 3)	3.36	2.96×10^{-13}
(Expt. 4)	2.46	3.30×10^{-13}
	Mean ± SD	4.02 ± 0.98

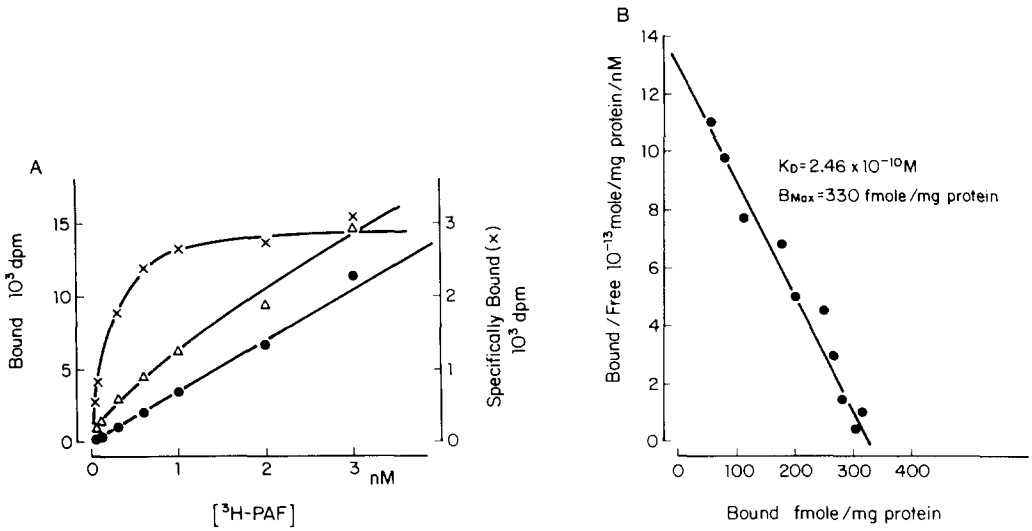

Fig. 2. (A) Binding of [^{3}H]PAF to human platelet membranes assayed in 10 mM $MgCl_2$, 10 mM Tris and 0.25% BSA, pH 7.0, as a function of [^{3}H]PAF concentration. One hundred micrograms of membrane protein was used for each tube. Each point is the mean of triplicates, and the standard deviation is less than 5% of the counts. Key: (●--●) nonspecific binding; (△--△) total binding; and (×--×) specific binding. Specific binding and nonspecific binding are defined in Materials and Methods. (B) Scatchard analysis of receptor binding data from Fig. 2A. In this particular experiment, [^{3}H]PAF binding to receptor in human platelet membranes had an equilibrium dissociation constant (K_D) of 2.46×10^{-10} M. The isolated human platelet membranes had a maximal detectable receptor site of 330 fmoles/mg membrane protein.

to be identical (within experimental error). As listed in Table 1, membrane fraction B contained more PAF receptor sites than membrane fraction A except for Memb #196. This difference between membrane fractions A and B was also observed in normal routine tests at a concentration of 1 nM [^{3}H]PAF; the specifically bound [^{3}H]PAF in membrane fraction B was higher than that in membrane fraction A. Also as shown in Table 1, the maximal number of PAF receptor sites seemed to vary from one membrane preparation to another. It varied from 80 to 500 fmole receptors per mg membrane protein. However, the K_D value seemed to remain quite constant (within experimental error). The equilibrium dissociation constant (mean ± standard deviation) calculated from those experimental results listed in Table 1 was 4.02 (±0.98) × 10^{-10} M. Membrane fraction B was the major portion of the membrane preparation and, therefore, was used throughout the experiments.

Inhibition of [^{3}H]*PAF receptor binding in human platelet membranes by PAF and its analogs and PAF*

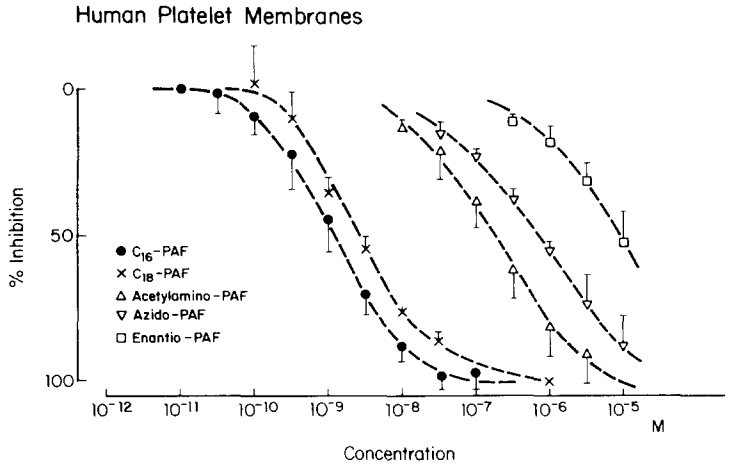

Fig. 3. Inhibition of specific [^{3}H]PAF binding to human platelet membranes by C_{18}-PAF (×--×), C_{16}-PAF (●--●), acetylamino-PAF (△--△), azido-PAF (▽--▽) and enantio-C_{16}-PAF (□--□). Each data point represents the average of three to four experiments and, in each experiment, triplicate determinations were performed.

Table 2. Equilibrium inhibition constant (K_i) of several selected PAF antagonists and PAF or PAF analogs to inhibit the binding of [^{3}H]PAF to rabbit platelet, human platelet or human lung membranes

Compound	K_i (M)		
	Rabbit platelets	Human platelets	Human lung tissues
L-651,142	$8.39 (\pm 6.5) \times 10^{-7}$	$1.82 (\pm 0.1) \times 10^{-6}$	$3.64 (\pm 0.23) \times 10^{-6}$
CV-3988	$4.13 (\pm 0.34) \times 10^{-7}$	$8.72 (\pm 3.2) \times 10^{-7}$	$2.32 (\pm 0.47) \times 10^{-6}$
Gingkolide B	$2.23 (\pm 0.76) \times 10^{-7}$	$3.65 (\pm 0.67) \times 10^{-7}$	$5.24 (\pm 1.40) \times 10^{-7}$
L-652,731	$9.83 (\pm 2.92) \times 10^{-9}$	$1.03 (\pm 0.15) \times 10^{-7}$	$1.66 (\pm 0.35) \times 10^{-7}$
L-653,150	$2.00 (\pm 1.16) \times 10^{-8}$	$8.91 (\pm 2.0) \times 10^{-8}$	$1.04 (\pm 0.66) \times 10^{-7}$
Kadsurenone	$8.81 (\pm 1.4) \times 10^{-8}$	$1.43 (\pm 0.24) \times 10^{-7}$	$1.85 (\pm 0.69) \times 10^{-7}$
Ono-6240	$1.11 (\pm 0.50) \times 10^{-7}$	$4.86 (\pm 1.44) \times 10^{-8}$	$8.9 (\pm 6.7) \times 10^{-8}$
C_{16}-PAF	$5.37 (\pm 0.32) \times 10^{-10}$	$4.10 (\pm 0.56) \times 10^{-10}$	$1.27 (\pm 0.35) \times 10^{-10}$
C_{18}-PAF	$1.06 (\pm 0.14) \times 10^{-9}$	$7.20 (\pm 0.52) \times 10^{-10}$	$5.86 (\pm 2.21) \times 10^{-10}$
Acetylamino-PAF	$9.78 (\pm 2.1) \times 10^{-8}$	$5.08 (\pm 4.11) \times 10^{-8}$	$2.87 (\pm 1.86) \times 10^{-8}$
Azido-PAF	$3.60 (\pm 1.4) \times 10^{-7}$	$3.05 (\pm 1.89) \times 10^{-7}$	$1.86 (\pm 1.13) \times 10^{-7}$
Enantio-C_{16}-PAF	$3.51 (\pm 0.7) \times 10^{-6}$	$4.49 (\pm 2.55) \times 10^{-6}$	$1.98 (\pm 1.58) \times 10^{-6}$

Values are means ± SD; N = 3–5.

receptor antagonists. Figure 3 shows the normalized inhibition of [^{3}H]PAF receptor binding in human platelet membranes by C_{16}-PAF, C_{18}-PAF, acetylamino-PAF, azido-PAF and enantio-C_{16}-PAF. Similar to results found for rabbit platelet membranes, C_{16}-PAF was more potent than C_{18}-PAF with an EC_{50} of 1.0×10^{-9} M and 2.8×10^{-9} M respectively. Enantio-C_{16}-PAF was about 10,000 times less potent than C_{16}-PAF. Acetylamino-PAF and azido-PAF had EC_{50} values of 1.7×10^{-7} and 7.6×10^{-7} M respectively. The equilibrium inhibition constants (K_i) calculated from the

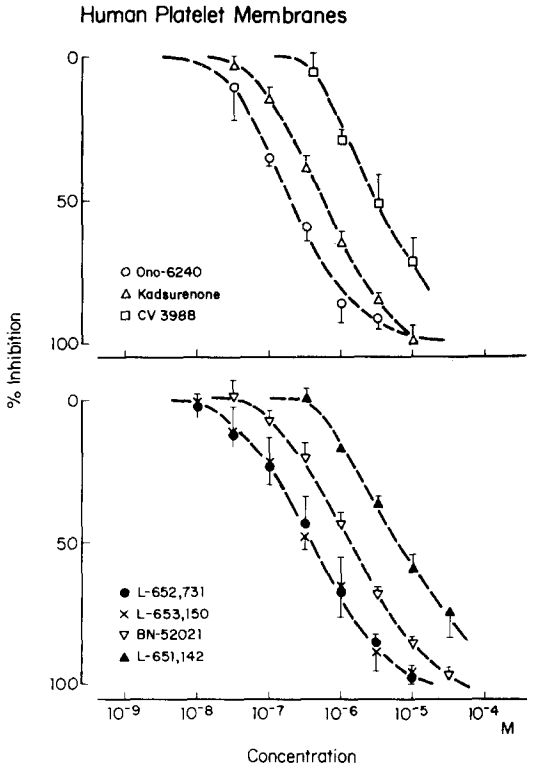

Fig. 4. Inhibition of specific [^{3}H]PAF binding to human platelet membranes by PAF receptor antagonists. Key: Ono-6240 (○--○); kadsurenone (△--△) L-652,731 (●--●); L-653,150 (×--×); ginkgolide B (▽--▽); CV-3988 (□--□) and L-651,142 (▲--▲). Each point represents the average of three to four experiments and, in each experiment, triplicate determinations were performed.

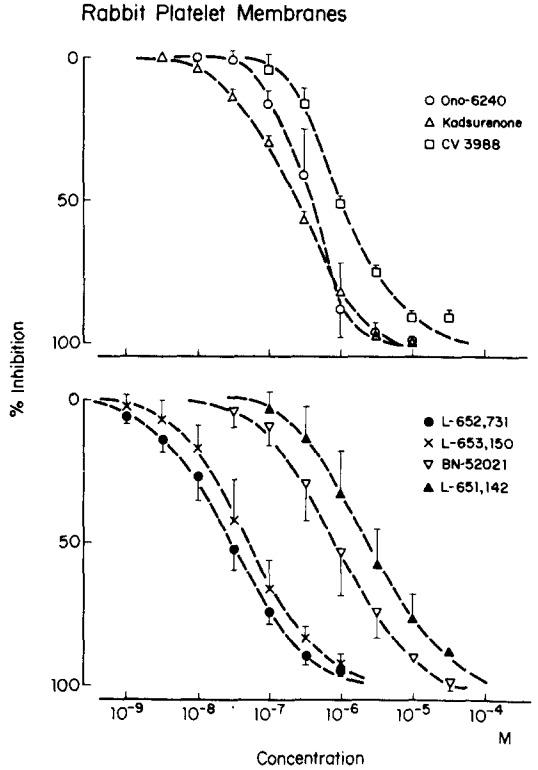

Fig. 5. Inhibition of specific [^{3}H]PAF binding to rabbit platelet membranes by PAF receptor antagonists. Key: Ono-6240 (○--○); kadsurenone (△--△); L-652,731 (●--●); L-653,150 (×--×); ginkgolide B (▽--▽); CV-3988 (□--□) and L-651,142 (▲--▲). Each data point represents the average of two to three experiments and, in each experiment, duplicate determinations were performed.

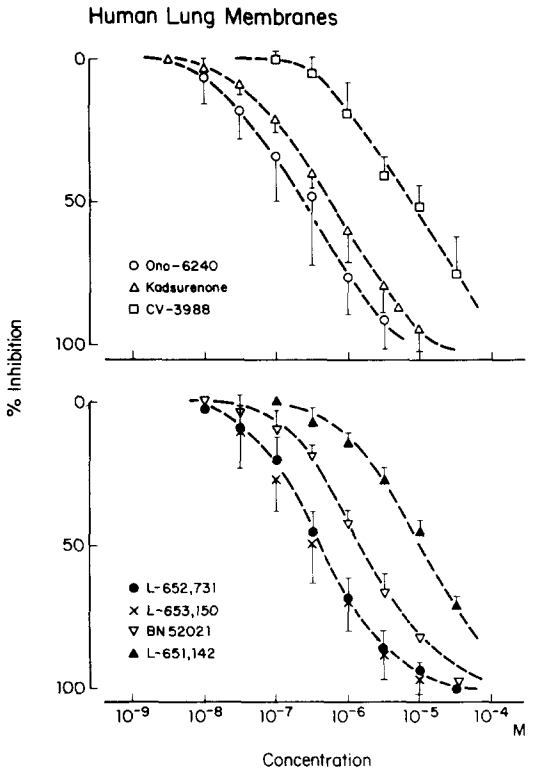

Fig. 6. Inhibition of specific [^{3}H]PAF binding to membrane fragments of human lung tissues by Ono-6240 (○--○), L-653,150 (×—×), kadsurenone (△--△), L-652,731 (●--●), ginkgolide B (▽--▽), CV-3988 (□--□) and L-651,142 (▲--▲). Each data point represents the average of three to four experiments and, in each experiment, triplicate determinations were performed.

Cheng and Prusoff equation [27] after the IC_{50} values were properly determined from the indirect Hill plot, [26] are listed in Table 2. The relative potencies and the K_i values of PAF and PAF analogs in human platelets were quite similar to those in rabbit platelets and human lung tissues.

Figure 4 shows the inhibitory results of [^{3}H]PAF receptor binding to human platelet membranes by the receptor antagonists shown in Fig. 1. The relative potencies of these PAF receptor antagonists in human platelets were as follows: Ono-6240 > L-652,731 = L-653,150 ≈ kadsurenone > ginkgolide B > CV-3988 > L-651,142. Ono-6240 was the most potent one in human platelets with an EC_{50} of 2.1×10^{-7} M (Fig. 4) and a K_i of 4.86 ($\pm$1.44) $\times$ 10^{-8} M (see Table 2).

Inhibition of [^{3}H]*PAF receptor binding to rabbit platelet membranes.* Figure 5 shows the percent inhibition of [^{3}H]PAF binding to specific receptors in rabbit platelet membranes. As shown in Fig. 5 and Table 2, the relative potencies of these PAF receptor antagonists in rabbit platelet membranes followed the order of L-652,731 > L-653,150 > kadsurenone ≈ Ono-6240 > ginkgolide B > CV-3988 > L-651,142. L-652,731 and L-653,150 showed roughly the same potency in rabbit platelets, followed by Ono-6240 and kadsurenone, both of which had a K_i of $\sim 1 \times 10^{-7}$ M which is about five to ten times less potent than that of L-652,731 [$K_i = 9.83$ ($\pm$2.92) $\times$ 10^{-9} M]. CV-3988 and ginkgolide B showed roughly the same potency with EC_{50} values of 7.7×10^{-7} M and 1.0×10^{-6} M respectively. L-651,142 was the least potent one on the list with an EC_{50} of 2.3×10^{-6} M (Fig. 5).

Inhibition of [^{3}H]*PAF binding to membrane fragments of human lung tissues.* Figure 6 shows the percent inhibition of [^{3}H]PAF binding to membrane fragments of human lung tissues. As shown in Fig. 6 and the calculated K_i values listed in Table 2, the relative potencies of these PAF receptor antagonists were as follows: Ono-6240 > L-653,150 ≈ L-652,731 ≈ kadsurenone > ginkgolide B > CV-3988 > L-651,142. Ono-6240 was the most potent one with $K_i = 8.9$ ($\pm$6.7) $\times$ 10^{-8} M.

DISCUSSION

From the equilibrium inhibition constants (K_i) listed in Table 2, most of the compounds tested including PAF receptor agonists and antagonists showed roughly identical potencies to inhibit the [^{3}H]PAF binding to either rabbit or human platelet membranes. However, Ono-6240 was about two times more potent in human platelets. On the other hand, L-652,731 and its thiophene analog (L-653,150) showed five to ten times less potency in human platelets. The significant species difference in the potency of L-652,731 or L-653,150 was clearly demonstrated in an indirect Hill plot (Figs. 7 and 8). The straight line for human platelets obtained from

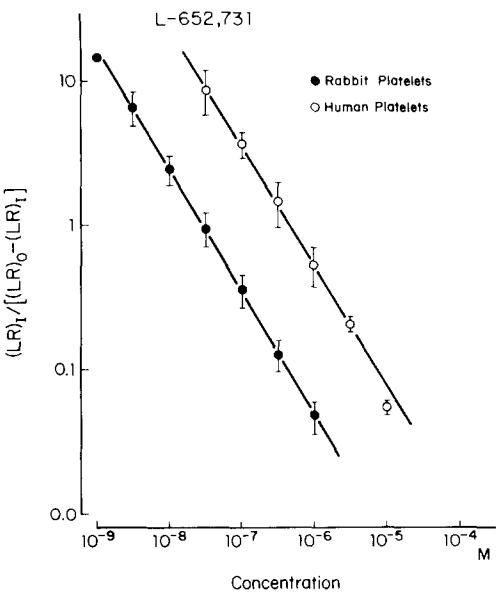

Fig. 7. Indirect Hill plots of the inhibitory results on the specific [^{3}H]PAF binding to rabbit and human platelets by L-652,731. $(LR)_0$ is the amount of [^{3}H]PAF specific bound in the absence of competitive inhibitor; $(LR)_t$ is the amount of specifically bound in the presence of inhibitor. Since the slope is close to -1, the intercept on the abscissa equals the IC_{50} value, which was 2.7×10^{-8} M in rabbit platelets (●--●) and 4.3×10^{-8} M in human platelets (○--○). The error bar represents the standard deviation.

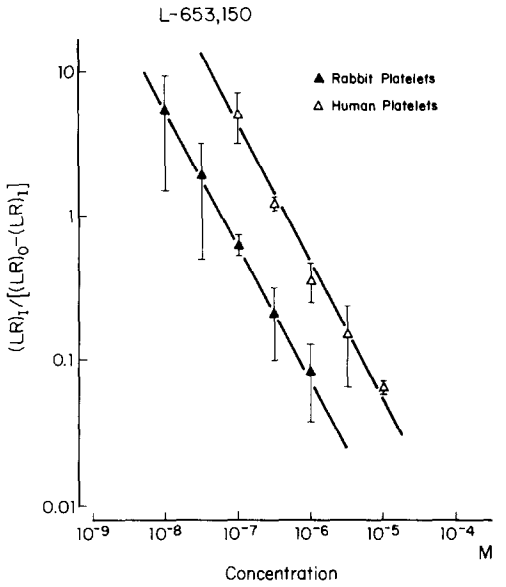

Fig. 8. Indirect Hill plots of the inhibitory results on the specific [^{3}H]PAF binding to rabbit (▲--▲) and human (△--△) platelets by L-653,150. See legend of Fig. 7 for definition of ordinate labeling. The IC_{50} values were 6.0×10^{-8} and 4.5×10^{-7} M in rabbit and human platelets respectively.

the linear regression of the data mean values is parallel but shifted to the right in both cases of L-652,731 and L-653,150.

In rabbit platelet membranes, L-652,731 had an equilibrium inhibition constant of 9.83×10^{-9} M which was about ten times more potent than that of kadsurenone ($K_i = 8.81 \times 10^{-8}$ M). However, in human platelet membranes, both compounds showed roughly the same potency when inhibiting the [^{3}H]PAF binding to the receptor, with a $K_i \sim 1 \times 10^{-7}$ M. Therefore, the affinity of L-652,731 for the receptors in rabbit platelet membranes was much greater than in human platelet membranes, whereas kadsurenone retained roughly the same affinity in both. L-652,731 [17], kadsurenone [14, 15], and L-653,150 (our unpublished results) have been shown to be PAF competitive receptor antagonists. These variations for L-652,731 and L-653,150 but not kadsurenone in potency between species strongly suggest that there is a species difference at or near the PAF binding site in the receptor of platelet activating factor even though the kadsurenone binding site remains intact during evolution. Differences in the molecular weight of PAF receptors for human and rabbit platelets were also reported recently. A human platelet PAF-binding protein with a molecular weight of 160,000 daltons has been isolated and purified [28, 29]. On the other hand, a 64 kD protein was reported from rabbit platelets based on the studies of the binding of a PAF analog with an iodinated photoreactive group at the ω-end of the long chain at position 1.*

Species differences in PAF-induced platelet functions have long been recognized. The most responsive platelets are found to be from guinea pigs, followed by those from rabbits, dogs and humans. Rat platelets in plasma are unresponsive to PAF. Such variations in sensitivity in different species seem to be due to differences in the number of receptor sites per cell but not in the equilibrium dissociation constant (K_D) of PAF to its own receptor. In our receptor binding studies, K_D values were found to be roughly identical, $5.3\ (\pm 0.6) \times 10^{-10}$ [22] and $4.0\ (\pm 0.98) \times 10^{-10}$ M for rabbit and human platelet membranes respectively. However, the detectable receptor sites in rabbit (2300 fmoles/mg membrane protein) [22] are about ten times more than those in human platelet membranes (100–500 fmoles/mg membrane protein). So far, we have not found any detectable [^{3}H]PAF specific binding from rat platelet membranes prepared following the same protocol used for human and rabbit platelet membranes.

In vivo, species differences in PAF-induced cutaneous vascular permeability were also reported among rats, guinea pigs and rabbits [14]; C_{18}-PAF is more potent than C_{16}-PAF in guinea pigs, whereas both are equally potent in rats. The PAF-induced plasma exudation in guinea pigs depends on cyclooxygenase activity in guinea pigs but not in rats. Furthermore, PAF or PAF analogs alone initiate the vascular response in either guinea pigs or rats. However, in rabbits, intradermal injection of PAF alone does not initiate vasopermeability; an intracutaneous coinjection of prostaglandin is always required to produce the plasma exudation [14, 30], even though a substantially vasoactive property has been reported for PAF intradermally injected alone in rabbits [31].

In humans, including platelets and lung tissues, the membrane preparations are always less reproducible than in rabbits. The maximal number of PAF receptor sites (B_{max}) as shown in Table 1, or the specifically bound [^{3}H]PAF to the same amount of membrane protein at a concentration of 1 nM [^{3}H]PAF, varied from one preparation to another. Whether this variation was due to sex, race or other factors is still unknown. For human lung membranes, different parts of the lung tissues may also play an important factor in this variation. However, the equilibrium dissociation constant seems to remain quite constant. Therefore, the relative potency or the calculated equilibrium inhibition constant (K_i) of the PAF receptor agonists or antagonists here should not be affected.

As found in other receptor systems, PAF may act through the inhibition of adenylate cyclase via a guanyl nucleotide regulatory protein in the rabbit platelet system [22, 32, 33]. The [^{3}H]PAF receptor binding in rabbits is inhibited specifically by Na^+ and GTP, but potentiated by divalent ions, such as Ca^{2+}, Mg^{2+} and Mn^{2+}. Both from the Scatchard analysis of [^{3}H]PAF saturable binding data and from the [^{3}H]PAF binding displacement curves with unlabeled PAF, PAF receptor is shown to be in the low affinity state in the presence of Na^+, whereas it is in the high affinity state in the presence of Mg^{2+}.

* P. Bette-Bobillo and A. Bienvenue, Third International Congress of Inflammation, Paris, Abstract 179 (1984).

However, for the PAF receptor antagonist L-652,731, patterns of [^{3}H]PAF binding displacement curves are totally reversed from those of PAF or PAF analogs [17]. The EC_{50} of the competition curve for L-652,731 is shifted from 3.5×10^{-8} M in the presence of 10 mM $MgCl_2$ to an EC_{50} of 6.3×10^{-9} M in the presence of 150 mM NaCl [17],whereas the competition curves for kadsurenone has been found to be insensitive to the ionic conditions [15, 22]. These results, in conjunction with the difference in affinity of L-652,731 but not of kadsurenone to human and rabbit platelets, strongly suggest that the binding points in the receptor for L-652,731 and kadsurenone are different even though both of them are competitive receptor antagonists, and thus they may share a common binding site in the PAF receptors.

Multiple molecular species of platelet activating factor have been reported recently [34–37]. However, whether they act on the same receptor or there exist subtypes of PAF receptor remains to be elucidated. Here, we compared the inhibition of [^{3}H]PAF receptor binding to membranes from human platelets and human lung tissues by a few PAF analogs and several PAF receptor antagonists. No dramatic difference was found in the potencies of PAF receptor agonists and antagonists when inhibiting [^{3}H]PAF receptor binding to either human platelet or human lung tissue membranes even though C_{16}-PAF showed slightly higher potency in human lung but CV-3988 and Ono-6240 showed slightly less. Therefore, from results demonstrated here, there seems no clear evidence that there is a difference in the PAF receptors between human platelets and human lung tissues. However, a possible existence of PAF receptor subtypes has been proposed from the difference between the potencies of kadsurenone when inhibiting the PAF-induced chemiluminescence of guinea pig peritoneal macrophages and the leukocyte aggregation in guinea pig peripheral blood [38]. It will be interesting to see the difference in the [^{3}H]PAF binding and the binding inhibition in these two cell types.

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